10/759,277

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004
L1        185254 S SYNTHETASE?
L2             1 S "CMP SILAIC ACID"
L3            11 S "SILAIC ACID"
L4             0 S L1 AND L3
L5         69303 S "SIALIC ACID"
L6           603 S L1 AND L5
L7         21033 S "CMP"
L8           438 S L6 AND L7
L9       6727337 S CLON? OR EXPRESS? OR RECOMBINANT
L10          182 S L8 AND L9
                 E COLEMAN T A/AU
L11          214 S E3
                 E BETENBAUGH M J/AU
L12          412 S E3-E7
L13          613 S L11 OR L12
L14            7 S L10 AND L13
L15            4 DUP REM L14 (3 DUPLICATES REMOVED)
L16           29 S HUMAN AND L10
L17           22 DUP REM L16 (7 DUPLICATES REMOVED)

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 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  Jul 12   BEILSTEIN enhanced with new display and select options,
                   resulting in a closer connection to BABS
 NEWS  4  AUG 02   IFIPAT/IFIUDB/IFICDB reloaded with new search and display
                   fields
 NEWS  5  AUG 02   CAplus and CA patent records enhanced with European and Japan
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                   status data from INPADOC
 NEWS  9  SEP 01   INPADOC: New family current-awareness alert (SDI) available
 NEWS 10  SEP 01   New pricing for the Save Answers for SciFinder Wizard within
                   STN Express with Discover!
 NEWS 11  SEP 01   New display format, HITSTR, available in WPIDS/WPINDEX/WPIX
 NEWS 12  SEP 14   STN Patent Forum to be held October 13, 2004, in Iselin, NJ
 NEWS 13  SEP 27   STANDARDS will no longer be available on STN
 NEWS 14  SEP 27   SWETSCAN will no longer be available on STN
 NEWS 15  SEP 30   STN downtime scheduled October 2-3, 2004

 NEWS EXPRESS  JULY 30 CURRENT WINDOWS VERSION IS V7.01, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
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                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

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FILE 'LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)


=> s synthetase?
L1      185254 SYNTHETASE?

=> s "CMP silaic acid"
L2            1 "CMP SILAIC ACID"

=> d all

L2   ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2004 The Thomson Corporation.  on STN
AN   1984:218230  BIOSIS
DN   PREV198477051214; BA77:51214
TI   DEFICIENT CMP N ACETYL NEURAMINIC-ACID GLYCO PROTEIN SIALYL TRANSFERASE
     ACTIVITY IN A CLONE OF KB CELLS WITH ALTERED CELL FUSION ABILITY.
AU   TOYAMA S [Reprint author]; KOYAMA A H; TOYAMA S
CS   INST VIRUS RES, KYOTO UNIV, KYOTO 606, JAPAN
SO   Journal of Biological Chemistry, (1983) Vol. 258, No. 15, pp. 9147-9152.
     CODEN: JBCHA3. ISSN: 0021-9258.
DT   Article
FS   BA
LA   ENGLISH
AB   Lines of KB cells resistant to Sendai virus-induced cytolysis were
     isolated and characterized.  The nature of this mutation was studied.
     Plasma membrane fractions from Sil cells had decreased amount of sialic
     acid and the same amount of galactose as compared to the membranes from
     parental KB cells.  Sil cells exhibited an increase in sensitivity to
     toxic effects of ricin and a decrease in sensitivity to wheat germ
     agglutinin.  Binding of wheat germ agglutinin to Sil cells was markedly
     decreased.  Several membrane glycoproteins of Sil cells migrated slightly
     faster than the corresponding bands of wild type membrane when examined by
     gel electrophoresis in sodium dodecyl sulfate.  Sil cells had decreased
     sialytransferase activity that catalyzed the transfer or sialic acid
```

residues from CMP-N-acetylneuraminic acid to glycoprotein acceptors containing Gaβ1 → 3GalNAcα1 → O-Ser(Thr) [β-galactosidase 1 → N-acetylgalactosamine → O-serine (threonine)] chain. The decreased enzyme activity could not be accounted for by the presence of inhibitors, altered pH optimum, or increased sialidase or **CMP-silaic acid** hydrolase activities. A molecular basis for the Sil cell phenotype might be the deficiency of sialytransferase.

CC Cytology - Human 02508
Genetics - Human 03508
Biochemistry methods - Proteins, peptides and amino acids 10054
Biochemistry methods - Carbohydrates 10058
Biochemistry studies - General 10060
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Biochemistry studies - Proteins, peptides and amino acids 10064
Biochemistry studies - Carbohydrates 10068
Biophysics - Methods and techniques 10504
Biophysics - Membrane phenomena 10508
Enzymes - Physiological studies 10808
Movement 12100
Metabolism - Carbohydrates 13004
Metabolism - Proteins, peptides and amino acids 13012
Metabolism - Nucleic acids, purines and pyrimidines 13014
Dental biology - General and methods 19001
Toxicology - General and methods 22501
Neoplasms - Neoplastic cell lines 24005
Virology - Animal host viruses 33506
Plant physiology - Chemical constituents 51522
Agronomy - Grain crops 52504

IT Major Concepts
Cell Biology; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Toxicology; Tumor Biology

IT Miscellaneous Descriptors
HUMAN ORAL EPIDERMOID CARCINOMA KB CELL HUMAN ORAL EPIDERMOID CARCINOMA SIL CELL SENDAI VIRUS INDUCED CYTOLYSIS SIALIDASE CMP SIALIC-ACID HYDROLASE RICIN WHEAT GERM AGGLUTININ MEMBRANE GLYCO PROTEIN SIALIC-ACID GALACTOSE GENETIC ENGINEERING

ORGN Classifier
Paramyxoviridae 03503
Super Taxa
Negative Sense ssRNA Viruses; Viruses; Microorganisms
Taxa Notes
Microorganisms, Negative Sense Single-Stranded RNA Viruses, Viruses

ORGN Classifier
Gramineae 25305
Super Taxa
Monocotyledones; Angiospermae; Spermatophyta; Plantae
Taxa Notes
Angiosperms, Monocots, Plants, Spermatophytes, Vascular Plants

ORGN Classifier
Euphorbiaceae 26055
Super Taxa
Dicotyledones; Angiospermae; Spermatophyta; Plantae
Taxa Notes
Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants

ORGN Classifier
Tupaiidae 86245
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates, Nonhuman Primates, Primates, Vertebrates

RN 3063-71-6 (CMP-N-ACETYLNEURAMINIC-ACID)
321976-25-4 (SIALYLTRANSFERASE)

```
     9001-67-6 (SIALIDASE)
     55326-41-5 (CMP-SIALIC-ACID HYDROLASE)
     59-23-4Q (GALACTOSE)
     26566-61-0Q (GALACTOSE)
     9075-81-4 (SIALYL TRANSFERASE)
     50855-33-9Q (GALACTOSE)


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=> s "silaic acid"
L3            11 "SILAIC ACID"

=> s l1 and l3
L4             0 L1 AND L3

=> s "sialic acid"
L5         69303 "SIALIC ACID"

=> s l1 and l5
L6           603 L1 AND L5

=> s "CMP"
L7         21033 "CMP"

=> s l6 and l7
L8           438 L6 AND L7

=> s clon? or express? or recombinant
   5 FILES SEARCHED...
L9       6727337 CLON? OR EXPRESS? OR RECOMBINANT

=> s l8 and l9
L10          182 L8 AND L9

=> e coleman t a/au
E1             1     COLEMAN SYLVIA ETHEL/AU
E2           382     COLEMAN T/AU
E3           214 --> COLEMAN T A/AU
E4             5     COLEMAN T B/AU
E5             7     COLEMAN T C/AU
E6            15     COLEMAN T D/AU
E7            16     COLEMAN T E/AU
E8            52     COLEMAN T F/AU
E9           414     COLEMAN T G/AU
E10          136     COLEMAN T H/AU
E11            1     COLEMAN T H JR/AU
E12          105     COLEMAN T J/AU

=> s e3
L11          214 "COLEMAN T A"/AU

=> e betenbaugh m j/au
E1             1     BETENBAUGH H S/AU
E2            16     BETENBAUGH M/AU
E3           245 --> BETENBAUGH M J/AU
E4             2     BETENBAUGH M J */AU
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E5          14      BETENBAUGH MICHAEL/AU
E6         150      BETENBAUGH MICHAEL J/AU
E7           1      BETENBAUGH MICHAEL JOSEPH/AU
E8           3      BETENBAUGH MJ/AU
E9           3      BETENBAUGH T M/AU
E10          5      BETENCOURT A/AU
E11          3      BETENCOURT ALAIN/AU
E12          1      BETENCOURT J C A/AU

=> s e3-e7
L12        412 ("BETENBAUGH M J"/AU OR "BETENBAUGH M J *"/AU OR "BETENBAUGH
               MICHAEL"/AU OR "BETENBAUGH MICHAEL J"/AU OR "BETENBAUGH MICHAEL
               JOSEPH"/AU)

=> s l11 or l12
L13        613 L11 OR L12

=> d his

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
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L5       69303 S "SIALIC ACID"
L6         603 S L1 AND L5
L7       21033 S "CMP"
L8         438 S L6 AND L7
L9     6727337 S CLON? OR EXPRESS? OR RECOMBINANT
L10        182 S L8 AND L9
               E COLEMAN T A/AU
L11        214 S E3
               E BETENBAUGH M J/AU
L12        412 S E3-E7
L13        613 S L11 OR L12

=> s l10 and l13
L14          7 L10 AND L13

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15          4 DUP REM L14 (3 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L15   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-06688  BIOTECHDS
TITLE:            Manipulating glycoprotein production in insect cell, involves
                  enhancing expression of enzymes involved in
                  carbohydrate processing pathway such as N-acetylglucosamine-2
                  epimerase or sialic acid
                  synthetase;
                       recombinant protein production via plasmid
                     expression in host cell for use in diagnosis and
                     therapy
AUTHOR:           BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T
                  A
PATENT ASSIGNEE:  BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A
PATENT INFO:      US 2002142386 3 Oct 2002
APPLICATION INFO: US 2001-930440 16 Aug 2001
PRIORITY INFO:    US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999
```

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-102519 [09]

AB DERWENT ABSTRACT:

NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAc, **sialic acid synthetase**, aldolase, cytidine monophosphate-**sialic acid** (**CMP**-SA) **synthetase** or **CMP**-SA transporter, where the **expression** of each (E) is enhanced to above endogenous levels, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP**-SA above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP**-SA, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.

WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B) sequence variants of the amino acid sequence or nucleotide sequence of human aldolase, human **CMP**-SA **synthetase** or SA-**synthetase**, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP**-SA **synthetase** is a human enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na+, K+-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP**-SA **synthetase**. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na+, K+-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics

and therapeutics.
EXAMPLE - No suitable example given. (88 pages)

L15 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2001-14234 BIOTECHDS
TITLE: Cells producing cytidine monophosphate-**sialic acid** and sialylated glycoprotein above endogenous levels for production of vaccines and therapeutics; metabolic engineering for **recombinant** vaccine production
AUTHOR: **Betenbaugh M J**; Lawrence S; Lee Y C; **Coleman T A**; Palter K; Jarvis D
PATENT ASSIGNEE: Hum.Genome-Sci.; Univ.Johns-Hopkins; Univ.Temple; Univ.Wyoming
LOCATION: Rockville, MD, USA; Baltimore, MD, USA; Philadelphia, PA, USA; Larame, WY, USA.
PATENT INFO: WO 2001042492 14 Jun 2001
APPLICATION INFO: WO 2000-US33136 7 Dec 2000
PRIORITY INFO: US 1999-169839 9 Dec 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-441575 [47]
AB Cells (C1 and C2) where C1 produces the donor substrate cytidine monophosphate-**sialic acid** (**CMP**-SA) above endogenous levels and C2 produces sialylated glycoprotein above endogenous levels, are claimed. Also claimed are: a kit for **expressing** sialylated glycoproteins comprising C1 or C2; manipulating glycoprotein production in an insect cell involving enhancing N-acetylglucosamine-2-epimerase, an enzyme catalyzing conversion of UDP-N-acetylglucosamine to N-**CMP**-SA-**synthetase** or **CMP**-SA-transporter above endogenous levels; producing sialylated glycoproteins comprising **expressing** a heterologous protein in an insect cell manipulated according to the method; producing sialylated glycoprotein in a cell by determining the carbohydrate substrates in a cell, transforming the cell with enzymes to give necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein. The glycoproteins are useful in vaccines and as diagnostic tools. (182pp)

L15 ANSWER 3 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
ACCESSION NUMBER: 2001412468 EMBASE
TITLE: **Cloning** and **expression** of human **sialic acid** pathway genes to generate **CMP-sialic acids** in insect cells.
AUTHOR: Lawrence S.M.; Huddleston K.A.; Tomiya N.; Nguyen N.; Lee Y.C.; Vann W.F.; **Coleman T.A.**; **Betenbaugh M.J.**
CORPORATE SOURCE: M.J. Betenbaugh, Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, United States. beten@jhu.edu
SOURCE: Glycoconjugate Journal, (2001) 18/3 (205-213).
Refs: 38
ISSN: 0282-0080 CODEN: GLJOEW
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The addition of **sialic acid** residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar

nucleotide cytidine monophospho-**sialic acid** (**CMP**-SA) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, **CMP**-N-acetylneuraminic acid (**CMP**-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for **CMP**-SA synthesis have been engineered into insect cells using the baculovirus **expression** system. In this study, a human **CMP-sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The human **CMP-sialic acid** synthase (**Cmp**-Sas) is ubiquitously **expressed** in human cells from multiple tissues. When **expressed** in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-**expression** of **Cmp**-Sas with the recently **cloned** **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular **CMP**-Neu5Ac levels 30 times higher than those observed in unsupplemented CHO cells. The absence of any one of these three components abolishes **CMP**-Neu5Ac production in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, **CMP**-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (**CMP**-KDN), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human **CMP**-SAS enzyme is also capable of **CMP**-N-glycolylneuraminic acid (**CMP**-Neu5Gc) synthesis when provided with the proper substrate. Engineering the **CMP**-SA metabolic pathway may be beneficial in various cell lines in which **CMP**-Neu5Ac production limits sialylation of glycoproteins or other glycans.

L15 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 3

ACCESSION NUMBER: 2000-14572 BIOTECHDS
TITLE: **Recombinant** production of sialylated glycoproteins using cells in which the **expression** of enzyme, e.g. **sialic acid-synthetase**, involved in the sialylation reaction has been altered; production of sialylated glycoprotein
AUTHOR: **Betenbaugh M J**; Lawrence S; Lee Y C; Jarvis D; **Coleman T A**
PATENT ASSIGNEE: Hum.Genome-Sci.; Univ.Johns-Hopkins; Univ.Wyoming
LOCATION: Rockville, MD, USA; Baltimore, MD, USA; Laramie, WY, USA.
PATENT INFO: WO 2000052135 8 Sep 2000
APPLICATION INFO: WO 2000-US5313 1 Mar 2000
PRIORITY INFO: US 990169624 8 Dec 1999; US 1999-122582 2 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-572178 [53]

AB Methods and recombinantly engineered cells for producing glycoproteins having sialylated oligosaccharides is claimed. The methods involve altering the **expression** of enzymes involved in carbohydrate processing e.g. **sialic-acid-synthetase**.
Also claimed are: a cell (I) producing the donor substrate cytidine monophosphate-**sialic acid** (**CMP**-SA) above endogenous levels; a kit (II) for **expression** of sialylated glycoprotein, containing (I); a method (III) for manipulating glycoprotein production in an insect cell by enhancing **expression** of at least one enzyme; a method (IV) for producing sialylated glycoprotein by **expressing** a heterologous protein in an insect cell manipulated via (III); and a method (V) for producing a sialylated glycoprotein in a cell of interest by determining the carbohydrate substances in the cell, transforming the cell with enzyme to produce necessary precursor substrates, and constructing a processing pathway in

the cell to produce a sialylated glycoprotein. The methods and cells may be used for producing sialylate glycoproteins. (144pp)

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L8           438 S L6 AND L7
L9       6727337 S CLON? OR EXPRESS? OR RECOMBINANT
L10          182 S L8 AND L9
                 E COLEMAN T A/AU
L11          214 S E3
                 E BETENBAUGH M J/AU
L12          412 S E3-E7
L13          613 S L11 OR L12
L14            7 S L10 AND L13
L15            4 DUP REM L14 (3 DUPLICATES REMOVED)

=> s human and l10
L16          29 HUMAN AND L10

=> dup rem l16
PROCESSING COMPLETED FOR L16
L17           22 DUP REM L16 (7 DUPLICATES REMOVED)

=> d 1-22 ibib ab

L17   ANSWER 1 OF 22  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-18268  BIOTECHDS
TITLE:            Producing glycoprotein with animal type sugar chain,
                  comprises introducing gene encoding enzyme that adds
                  sialic acid to non-reducing terminal of
                  sugar chain, and gene of heterologous protein, into plant
                  cell, cultivating plant cell;
                     transgenic plant construction via bacterium-mediated
                     transformation for use in protein production
AUTHOR:           FUJIYAMA K; SEKI T
PATENT ASSIGNEE:  FUJIYAMA K; SEKI T
PATENT INFO:      WO 2004063370 29 Jul 2004
APPLICATION INFO: WO 2004-P 264 15 Jan 2004
PRIORITY INFO:    JP 2003-7687 15 Jan 2003; JP 2003-7687 15 Jan 2003
DOCUMENT TYPE:    Patent
LANGUAGE:         Japanese
OTHER SOURCE:     WPI: 2004-561900 [54]
AB    DERWENT ABSTRACT:
      NOVELTY - Producing (M1) glycoprotein (I) having animal type sugar chain,
      involves introducing a gene encoding the enzyme that can add
      sialic acid to the non-reducing terminal of sugar
      chain, and the gene of heterologous protein, into the plant cell,
      cultivating the transformed plant cell, and recovering the culture
      solution of the plant cell.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
      following: (1) plant cell (II) transformed with the gene that codes
      sialic acid synthetase, CMP-
```

sialic acid synthetase and/or **CMP-sialic acid** transporter exhibiting saccharide addition mechanism (adding **sialic acid** to the non-reducing terminal of the sugar chain of glycoprotein), where (II) can take in the precursor of **sialic acid** or **sialic acid**, and has a vesicle that allows the uptake of **sialic acid**; and (2) plant body regenerate from (II).

BIOTECHNOLOGY - Preferred Method: In (M1), the glycoprotein having animal type sugar chain contains a core sugar chain and the external sugar chain, where the core sugar chain essentially comprises several numbers of mannose and acetylglucosamine, while the external sugar chain has a terminal sugar chain moiety containing a non-reducing end galactose. The external sugar chain is the linear or mono, tri or tetra branched sugar chain.

USE - (M1) is useful for producing glycoprotein having animal type sugar chain (claimed).

EXAMPLE - The DNA of the Escherichia coli was used as the template, PCR was performed using the primers having the sequences such as 5'-tttagctcgagacaatgagtaatatatatat-3' and 5'-tttttctcgagttattattccccctgatttttaaattc-3', the obtained PCR product was digested by XhoI and SalI restriction enzymes, and the neuB fragment was obtained. The DNA of the Nicotiana tabacum cv SRI was used as the template, PCR was performed using the primers having the sequences such as 5'-tttaagtcgacacgatgagagg-3' and 5'-aatcgtcgacccttaactgtc-3', the obtained PCR product was digested by restriction enzymes. The obtained neuB fragment, and the **CMP-sialic acid** transporter (CST) gene were connected, and the target CTS-neuB gene was obtained. The obtained CTS-neuB gene was introduced into the plasmid pBI221, and the vector pBI121-CTS-neuB was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pBI121-CTS-neuB by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of CTS-neuB gene was obtained. The cDNA of the **human** kidney was used as the template, PCR was performed using the primers having the sequences such as 5'-gttactagtatggactcggtggagaaggggccgccacctccgtcctcaacccgcggggcgaccgtccc-3' and 5'-tgggagctcctatttttggcatgaattatt-3', the obtained PCR product was introduced into the plasmid pBI221. The obtained **expression** cassette was then introduced into pGPTV-HPT, and the pGPTV-HPT-hCSS was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pGPTV-HPT-hCSS by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of HPT-hCSS gene was obtained. The plasmid containing the **expression** cassette of HPT-hCST was also obtained. The plasmids containing the **expression** cassettes of CST-neuB gene and the HPT-hCST gene were digested by XhoI and SpeI enzymes, and both the XhoI/SpeI fragments were introduced into pGEM-T Easy vector, and the plasmid pGEM-T-hCST-CTS-neuB was obtained. To the plasmid pGEM-T-hCST-CTS-neuB gene, the **expression** cassette of HPT-hCSS was introduced, and the plasmid pGPTV-HPT-hCSS-hCST-CTS-neuB was obtained. The pGPTV-HPT-hCSS-hCST-CTS-neuB was introduced into Agrobacterium tumefaciens LBA4404. The transformed LBA4404 was allowed to infect the cell of tobacco. The infected tobacco cell was cultivated for 7 days, the DNA was isolated from the plant cell, and the presence of the **CMP-sialic acid synthetase** (CSS) and **CMP-sialic acid** transporter (CST) gene was detected. The results showed the presence of CSS and CST genes in the transformed plant cell. Thus the plant cell containing CST and CSS in its genome was obtained. (88 pages)

L17 ANSWER 2 OF 22 MEDLINE on STN
ACCESSION NUMBER: 2004336041 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15238249
TITLE: **CMP-sialic acid synthetase** of the nucleus.
AUTHOR: Kean Edward L; Munster-Kuhnel Anja K; Gerardy-Schahn Rita

CORPORATE SOURCE: Department of Ophthalmology, Case Western Reserve University, Cleveland, OH 4410, USA.
SOURCE: Biochimica et biophysica acta, (2004 Jul 6) 1673 (1-2) 56-65. Ref: 73
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 20040708
Last Updated on STN: 20040818
Entered Medline: 20040817

AB **Sialic acids** of cell surface glycoconjugates play a pivotal role in the structure and function of animal cells and in some bacterial pathogens. The pattern of cell surface sialylation is species specific, and, in the animal, highly regulated during embryonic development. A prerequisite for the synthesis of sialylated glycoconjugates is the availability of the activated sugar-nucleotide cytidine 5'-monophosphate N-acetylneuraminic acid (**CMP**-NeuAc), which provides the substrate for sialyltransferases. Trials to purify the enzymatic activity responsible for the synthesis of **CMP**-NeuAc from different animal sources demonstrated that the major localisation of the enzyme is the cell nucleus. These earlier findings were confirmed when the murine **CMP**-NeuAc **synthetase** was **cloned** and the subcellular transport of **recombinant** epitope tagged forms visualised by indirect immunofluorescence. Today, the primary sequence elements that direct murine **CMP**-NeuAc **synthetase** into the cell nucleus are known, however, information regarding the physiological relevance of the nuclear destination is still not available. With this article, we provide a detailed review on earlier and recent findings that identified and confirmed the unusual subcellular localisation of the **CMP**-NeuAc **synthetase**. In addition, we take the advantage to discuss most recent developments towards understanding structure--function relations of this enzyme.

L17 ANSWER 3 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:698614 HCAPLUS
DOCUMENT NUMBER: 139:228058
TITLE: **Human** genes and proteins involved regulation of angiogenesis and their use in drug screening, diagnosis, and therapy
INVENTOR(S): Colin, Sylvie; Schneider, Christophe; Al Mahmood, Salman
PATENT ASSIGNEE(S): Gene Signal, Fr.
SOURCE: Fr. Demande, 405 pp.
CODEN: FRXXBL
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2836686	A1	20030905	FR 2002-2717	20020304
FR 2836687	A1	20030905	FR 2002-4546	20020411
WO 2003074073	A2	20030912	WO 2003-FR695	20030304

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: FR 2002-2717 A 20020304
FR 2002-4546 A 20020411

AB **Human** genes and the corresponding proteins which are implicated in regulation of angiogenesis, antisense oligonucleotides complementary to these nucleic acids, antibodies to the proteins, and transgenic cells under- or overexpressing these genes are disclosed. The angiogenesis-related nucleic acids and proteins, antibodies, and transgenic cells **expressing** the angiogenesis-related nucleic acid may be used in diagnosis and therapy and in screening for angiogenesis-regulating compds. Vectors containing the angiogenesis-related nucleic acid and transgenic cells producing the encoded proteins are further disclosed. Thus, using a subtractive hybridization procedure, 54 genes the **expression** of which is altered during angiogenesis were identified.

L17 ANSWER 4 OF 22 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-06688 BIOTECHDS
TITLE: Manipulating glycoprotein production in insect cell, involves enhancing **expression** of enzymes involved in carbohydrate processing pathway such as N-acetylglucosamine-2 epimerase or **sialic acid synthetase**;
recombinant protein production via plasmid **expression** in host cell for use in diagnosis and therapy
AUTHOR: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A
PATENT ASSIGNEE: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A
PATENT INFO: US 2002142386 3 Oct 2002
APPLICATION INFO: US 2001-930440 16 Aug 2001
PRIORITY INFO: US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-102519 [09]
AB DERWENT ABSTRACT:
NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAc, **sialic acid synthetase**, aldolase, cytidine monophosphate-**sialic acid** (**CMP**-SA) **synthetase** or **CMP**-SA transporter, where the **expression** of each (E) is enhanced to above endogenous levels, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP**-SA above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP**-SA, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.
WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B)

sequence variants of the amino acid sequence or nucleotide sequence of **human** aldolase, **human CMP**-SA **synthetase** or SA-**synthetase**, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP**-SA **synthetase** is a **human** enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na+, K+-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP**-SA **synthetase**. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na+, K+-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics and therapeutics.

EXAMPLE - No suitable example given. (88 pages)

L17 ANSWER 5 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2001:435294 HCAPLUS
DOCUMENT NUMBER:	135:41800
TITLE:	**Recombinant** cells with altered intracellular sialylation pathways and their use in producing glycoproteins
INVENTOR(S):	Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Coleman, Timothy A.; Palter, Karen; Jarvis, Don
PATENT ASSIGNEE(S):	Human Genome Sciences, Inc, USA; Johns Hopkins University; Temple University; University of Wyoming
SOURCE:	PCT Int. Appl., 182 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001042492	A1	20010614	WO 2000-US33136	20001207

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-169839P P 19991209

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human sialic acid** 9-phosphate **synthetase** which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 6 OF 22 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2001467579 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11479279
TITLE: Molecular **cloning** of a unique **CMP-sialic acid synthetase** that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates.
AUTHOR: Nakata D; Munster A K; Gerardy-Schahn R; Aoki N; Matsuda T; Kitajima K
CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.
SOURCE: Glycobiology, (2001 Aug) 11 (8) 685-92. Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB027414
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010830
Last Updated on STN: 20011015
Entered Medline: 20011011

AB 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a **sialic acid** (Sia) that is ubiquitously **expressed** in vertebrates during normal development and tumorigenesis. Its **expression** is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including **CMP**-Sia **synthetase**. Using crude enzyme preparations, it was shown that mammalian **CMP**-Sia **synthetases** had very low activity to synthesize **CMP**-KDN from KDN and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP**-KDN and **CMP**-N-acetylneuraminic acid (Neu5Ac) (Terada et al. [1993] J. Biol. Chemical, 268, 2640-2648). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA **cloning** of trout **CMP**-Sia **synthetase** was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme.

Based on the Vmax/Km values, the **recombinant** trout enzyme had high activity toward both KDN and Neu5Ac (1.1 versus 0.68 min(-1)). In contrast, the **recombinant** murine enzyme had 15 times lower activity toward KDN than Neu5Ac (0.23 versus 3.5 min(-1)). Northern blot analysis suggested that several sizes of the mRNA are **expressed** in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one **cloned** enzyme has the ability to utilize both KDN and Neu5Ac as substrates efficiently and is useful for the production of **CMP**-KDN.

L17 ANSWER 7 OF 22 EMBASE
on STN DUPLICATE 2

ACCESSION NUMBER: 2001412468 EMBASE
TITLE: **Cloning** and **expression** of **human** **sialic acid** pathway genes to generate **CMP**-**sialic acids** in insect cells.
AUTHOR: Lawrence S.M.; Huddleston K.A.; Tomiya N.; Nguyen N.; Lee Y.C.; Vann W.F.; Coleman T.A.; Betenbaugh M.J.
CORPORATE SOURCE: M.J. Betenbaugh, Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, United States. beten@jhu.edu
SOURCE: Glycoconjugate Journal, (2001) 18/3 (205-213).
Refs: 38
ISSN: 0282-0080 CODEN: GLJOEW
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The addition of **sialic acid** residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (**CMP**-SA) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, **CMP**-N-acetylneuraminic acid (**CMP**-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for **CMP**-SA synthesis have been engineered into insect cells using the baculovirus **expression** system. In this study, a **human** **CMP**-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The **human** **CMP**-**sialic acid** synthase (**Cmp**-Sas) is ubiquitously **expressed** in **human** cells from multiple tissues. When **expressed** in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-**expression** of **Cmp**-Sas with the recently **cloned** **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular **CMP**-Neu5Ac levels 30 times higher than those observed in unsupplemented CHO cells. The absence of any one of these three components abolishes **CMP**-Neu5Ac production in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, **CMP**-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (**CMP**-KDN), is produced instead, indicating that alternative **sialic** **acid** glycoforms may eventually be possible in insect cells. The **human** **CMP**-SAS enzyme is also capable of **CMP**-N-glycolylneuraminic acid (**CMP**-Neu5Gc) synthesis when provided with the proper substrate. Engineering the **CMP**-SA metabolic pathway may be beneficial in various cell lines in which **CMP**-Neu5Ac production limits sialylation of glycoproteins or other glycans.

L17 ANSWER 8 OF 22 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2002:189008 BIOSIS
DOCUMENT NUMBER: PREV200200189008
TITLE: Sialylation of the Pasteurella multocida cell surface.
AUTHOR(S): Vimr, E. R. [Reprint author]; Lichtensteiger, C. A. [Reprint author]
CORPORATE SOURCE: University of Illinois at Urbana-Champaign, Urbana, IL, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 141. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Mar 2002
Last Updated on STN: 13 Mar 2002

AB Bacterial pathogens belonging to the Haemophilus-Acthinobacillus-Pasteurella (HAP) group are obligate microparasites of the mammalian oropharynx and can cause severe respiratory or invasive disease in **humans**, domestic animals, and wildlife. **Sialic acids** are ubiquitous components of mammalian cell surfaces and serum glycoconjugates. At least one HAP member, Haemophilus influenzae, has been shown to mimic the host environment by phase-variation of its surface **sialic acids**. To our knowledge, no other HAP member has been shown to sialylate its cell surface. However, recent DNA sequencing of the Pasteurella multocida genome suggests this bacterium may encode functions for **sialic acid** catabolism, activation (synthesis of **CMP-sialic acid**), and glycosyl transfer (a2,6-sialyltransferase). To determine if P. multocida is capable of sialylation, the cell-free membrane fractions from two common serotypes (types A and D) were shown to sialylate endogenous acceptor(s) when provided with exogenous **CMP**-(14C)**sialic acid**. Confirmation that the transferred **sialic acid** was incorporated into the expected glycoketosidic linkage was obtained by demonstrating sensitivity of the label to digestion with **recombinant** Vibrio cholerae sialidase. The predicted absence of the biosynthetic genes for **sialic acid** synthesis suggests, as we have shown previously for H. influenzae, that P. multocida acquires free **sialic acid** from its host and then makes a metabolic decision between catabolism or activation for cell surface sialylation. That P. multocida may synthesize two **CMP-sialic acid synthetases**, one of which is encoded by the last gene of a **sialic acid** catabolic operon, suggests this HAP bacterium enjoys considerable flexibility in its sialometabolism, potentially accounting for its wide host range.

L17 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:628244 HCAPLUS
DOCUMENT NUMBER: 133:218534
TITLE: **Human** glycosylation enzymes and cDNAs and their use in drug screening, diagnosis, and therapy
INVENTOR(S): Coleman, Timothy A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052136	A2	20000908	WO 2000-US5325	20000301
WO 2000052136	A3	20001228		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000033884	A5	20000921	AU 2000-33884	20000301
EP 1159406	A2	20011205	EP 2000-912096	20000301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6333182	B1	20011225	US 2000-516143	20000301
JP 2002537796	T2	20021112	JP 2000-602748	20000301
US 2002137175	A1	20020926	US 2001-984205	20011029
US 6783971	B2	20040831		
US 2004142442	A1	20040722	US 2004-759277	20040120

PRIORITY APPLN. INFO.:	US 1999-122409P	P	19990302
	US 2000-516143	A3	20000301
	WO 2000-US5325	W	20000301
	US 2001-984205	A3	20011029

AB The present invention relates to novel **human** glycosylation enzymes and isolated nucleic acids containing the coding regions of the genes encoding such enzymes. Also provided are vectors, host cells, antibodies, and **recombinant** methods for producing **human** glycosylation enzymes. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel **human** glycosylation enzyme polypeptides. Thus, a **human** cDNA encoding a protein with significant sequence homol. to mouse **CMP** N-acetylneuraminic acid **synthetase** was **cloned** and sequenced. This gene was **expressed** primarily in colon tissue. Another **human** cDNA encoded a protein with significant sequence homol. to C. jejuni cytidine **sialic acid synthetase**. A third **human** cDNA encoding a protein with significant sequence homol. to E. coli N-acetylneuraminic acid aldolase was **cloned** and sequenced. This gene was **expressed** primarily in immune cells and tissues such as primary dendritic cells, monocytes, and bone marrow.

L17 ANSWER 10 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2000:628243 HCAPLUS
DOCUMENT NUMBER:	133:233546
TITLE:	Engineering of intracellular sialylation pathways for sialylated glycoprotein production
INVENTOR(S):	Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Jarvis, Don; Coleman, Timothy A.
PATENT ASSIGNEE(S):	Human Genome Sciences, Inc., USA; Johns Hopkins University; University of Wyoming
SOURCE:	PCT Int. Appl., 145 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052135	A2	20000908	WO 2000-US5313	20000301
WO 2000052135	A3	20040108		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000035083	A5	20000921	AU 2000-35083	20000301
JP 2003524395	T2	20030819	JP 2000-602747	20000301
EP 1399538	A2	20040324	EP 2000-913684	20000301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
US 2002142386	A1	20021003	US 2001-930440	20010816
PRIORITY APPLN. INFO.:			US 1999-122582P P	19990302
			US 1999-169624P P	19991208
			WO 2000-US5313 W	20000301
			US 2000-227579P P	20000825

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human sialic acid** 9-phosphate **synthetase** which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

L17 ANSWER 11 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:374744 HCAPLUS
DOCUMENT NUMBER: 135:151270
TITLE: Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of Campylobacter jejuni
AUTHOR(S): Guerry, Patricia; Ewing, Cheryl P.; Hickey, Thomas E.; Prendergast, Martina M.; Moran, Anthony P.
CORPORATE SOURCE: Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, 20910, USA
SOURCE: Infection and Immunity (2000), 68(12), 6656-6662 CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three genes involved in biosynthesis of the lipooligosaccharide (LOS) core of Campylobacter jejuni MSC57360, the type strain of the HS:1 serotype, whose structure mimics GM2 ganglioside, have been **cloned** and characterized. Mutation of genes encoding proteins with homol. to a sialyl transferase (cstII) and a putative N-acetylmannosamine **synthetase** (neuC1), part of the biosynthetic pathway of N-acetylneuraminic acid (NeuNAc), have identical phenotypes. The LOS cores of these mutants display identical changes in electrophoretic mobility, loss of reactivity with cholera toxin (CT), and enhanced immunoreactivity with a hyperimmune polyclonal antiserum generated against whole cells of C. jejuni MSC57360. Loss of **sialic acid** in the core of the neuC1 mutant was confirmed by fast atom bombardment mass spectrometry. Mutation of a gene encoding a putative

β-1,4-N-acetylgalactosaminyltransferase (Cgt) resulted in LOS cores intermediate in electrophoretic mobility between that of wild type and the mutants lacking NeuNAc, loss of reactivity with CT, and a reduced immunoreactivity with hyperimmune antiserum. Chemical analyses confirmed the loss of N-acetylgalactosamine (GalNAc) and the presence of NeuNAc in the cgt mutant. These data suggest that the Cgt enzyme is capable of transferring GalNAc to an acceptor with or without NeuNAc and that the Cst enzyme is capable of transferring NeuNAc to an acceptor with or without GalNAc. A mutant with a nonsialylated LOS core is more sensitive to the bactericidal effects of **human** sera than the wild type or the mutant lacking GalNAc.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 12 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1999:149430 SCISEARCH
THE GENUINE ARTICLE: 166KQ
TITLE: Haemophilus ducreyi produces a novel sialyltransferase - Identification of the sialyltransferase gene and construction of mutants deficient in the production of the **sialic acid**-containing glycoform of the lipooligosaccharide
AUTHOR: Bozue J A; Tullius M V; Wang J; Gibson B W; Munson R S (Reprint)
CORPORATE SOURCE: CHILDRENS HOSP RES FDN, 700 CHILDRENS DR, ROOM W402, COLUMBUS, OH 43205 (Reprint); CHILDRENS HOSP RES FDN, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT PEDIAT, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT MED MICROBIOL, COLUMBUS, OH 43205; UNIV CALIF SAN FRANCISCO, DEPT PHARMACEUT CHEM, SAN FRANCISCO, CA 94143
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 FEB 1999) Vol. 274, No. 7, pp. 4106-4114.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 76

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Haemophilus ducreyi, the cause of the sexually transmitted disease chancroid produces a lipooligosaccharide (LOS) containing a terminal sialyl N-acetyllactosamine trisaccharide, Previously, we reported the identification and characterization of the N-acetylneuraminic acid cytidylsynthetase gene (neuA), Forty-nine base pairs downstream of the **synthetase** gene is an open reading frame (ORF) encoding a protein with a predicted molecular weight of 34,646, This protein has weak homology to the polysialyltransferase of Escherichia colt K92, Downstream of this ORF is the gene encoding the H, ducreyi homologue of the Salmonella typhimurium rmlB gene. Mutations were constructed in the neuA gene and the gene encoding the second ORF by insertion of an Omega kanamycin cassette, and isogenic strains were constructed. LOS was isolated from each strain and characterized by SDS-polyacrylamide gel electrophoresis, carbohydrate, and mass spectrometric analysis, LOS isolated from strains containing a mutation in neuA or in the second ORF, designated Ist, lacked the **sialic acid**-containing glycoform, Complementation studies were performed, The neuA gene and the ist gene were each **cloned** into the shuttle vector pLS88 after polymerase chain reaction amplification. Complementation of the mutation in the ist gene was observed, but we were unable to complement the neuA mutation, Since it is possible that transcription of the neuA gene and the Ist gene were coupled, we constructed a nonpolar mutation in the neuA

gene, in this construct, the neuA mutation was complemented, suggesting transcriptional coupling of the neuA gene and the ist gene, Sialyltransferase activity was detected by incorporation of C-14-labeled NeuAc from **CMP**-NeuAc into trichloroacetic acid-precipitable material when the Ist gene was overexpressed in the nonpolar neuA mutant. We conclude that the Ist gene encodes the H, ducreyi sialyltransferase, Since the Ist gene product has little, if any, structural relationship to other sialyltransferases, this protein represents a new class of sialyltransferase.

L17 ANSWER 13 OF 22 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000084724 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10619706
TITLE: Combinatorial PCR approach to homology-based **cloning**: **cloning** and **expression** of mouse and **human** GM3-synthase.
AUTHOR: Kapitonov D; Bieberich E; Yu R K
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Medical College of Virginia, Virginia Commonwealth University Richmond, 23298-0614, USA.
CONTRACT NUMBER: NS11853 (NINDS)
SOURCE: Glycoconjugate journal, (1999 Jul) 16 (7) 337-50. Journal code: 8603310. ISSN: 0282-0080.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000214

AB GM3-synthase, also known as sialyltransferase I (ST-I), catalyzes the transfer of a **sialic acid** residue from **CMP**-**sialic acid** onto lactosylceramide to form ganglioside GM3. In order to **clone** this enzyme, as well as other sialyltransferases, we developed an approach that we termed combinatorial PCR. In this approach, degenerate primers were designed on the basis of conserved sequence motifs of the ST3 family of sialyltransferases (STs). The nucleotide sequence of the primers was varied to cover all amino acid variations occurring in each motif. In addition, in some primers the sequence was varied to cover possible homologous substitutions that are absent in the available motifs. A panel of cDNA from 12 mouse and 8 **human** tissues was used to enable **cloning** of tissue- and stage-specific sialyltransferases. Using this approach, the fragments of 11 new putative sialyltransferases were isolated and sequenced so far. Analysis of the **expression** pattern of a particular sialyltransferase across the panel of cDNA from the different tissues provided information about the tissue specificity of ST **expression** . We chose two new ubiquitously **expressed human** and mouse STs to **clone** full-length copies and to assay for GM3-synthase activity. One of the STs, which exhibited the highest homology to ST3 Gal III, showed activity toward lactosylceramide (LacCer) and was termed ST3 Gal V according to the suggested nomenclature [1]. The other ubiquitously **expressed** sialyltransferase was termed ST3Gal VI. All isolated sialyltransferases were screened for alternatively spliced forms (ASF). Such forms were found for both **human** ST3Gal V and ST3Gal VI in **human** fetal brain cDNA library. The detailed **cloning** strategy, functional assay, and full length cDNA and protein sequences of GM3 synthase (ST3Gal V, or ST-I) are presented.

L17 ANSWER 14 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 96:508623 SCISEARCH

THE GENUINE ARTICLE: UV299
TITLE: PURIFICATION, **CLONING**, AND **EXPRESSION** OF A CYTIDINE 5'-MONOPHOSPHATE N-ACETYLNEURAMINIC ACID **SYNTHETASE** FROM HAEMOPHILUS-DUCREYI
AUTHOR: TULLIUS M V; MUNSON R S; WANG J; GIBSON B W (Reprint)
CORPORATE SOURCE: UNIV CALIF SAN FRANCISCO, SCH PHARM, DEPT PHARMACEUT CHEM, 926-S, 513 PARNASSUS AVE, SAN FRANCISCO, CA, 94143 (Reprint); UNIV CALIF SAN FRANCISCO, SCH PHARM, DEPT PHARMACEUT CHEM, SAN FRANCISCO, CA, 94143; OHIO STATE UNIV, CHILDRENS HOSP, RES FDN, COLUMBUS, OH, 43205; OHIO STATE UNIV, DEPT PEDIAT, COLUMBUS, OH, 43205; OHIO STATE UNIV, DEPT MED MICROBIOL & IMMUNOL, COLUMBUS, OH, 43205
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (28 JUN 1996) Vol. 271, No. 26, pp. 15373-15380.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 60

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An N-acetylneuraminic acid cytidylyltransferase (EC 2.7.7.43) (**CMP**-NeuAc **synthetase**) was isolated from a Haemophilus ducreyi strain 35000 cell lysate and partially characterized. The enzyme catalyzes the reaction of CTP and NeuAc to form **CMP**-NeuAc, which is the nucleotide sugar donor used by sialyltransferases. Previous studies have shown that the outer membrane lipooligosaccharides of H. ducreyi contain terminal **sialic acid** attached to N-acetyllactosamine and that this modification is likely important to its pathogenesis. Therefore, to investigate the role of **sialic acid** in a. ducreyi pathogenesis, the gene encoding the **CMP**-NeuAc **synthetase** was **cloned** using degenerate oligonucleotide probes derived from NH2-terminal sequence data, and the nucleotide sequence was determined. The derived amino acid sequence of the **CMP**-NeuAc **synthetase** gene has homology to other **CMP**-NeuAc **synthetases** and to a lesser extent to **CMP**-2-keto-3-deoxy-D-manno-octulosonic acid **synthetases**, The gene was **cloned** into a T7 **expression** vector, the protein **expressed** in Escherichia coli, and purified to apparent homogeneity by anion exchange, Green 19 dye, and hydrophobic interaction chromatography. The final step yielded 20 mg of pure protein/liter of culture, The protein has a predicted molecular mass of 25440.6 Pa, which was confirmed by electrospray mass spectrometry (M(expt) = 25439.9 +/- 1.4 Pa). The enzyme appears to exist as a dimer by size exclusion chromatography. In contrast to other bacterial **CMP**-NeuAc **synthetases**, the H. ducreyi enzyme exhibited a different substrate specificity, being capable of also using N-glycolylneuraminic acid as a substrate.

L17 ANSWER 15 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 96:752059 SCISEARCH
THE GENUINE ARTICLE: VL560
TITLE: THE BIOCHEMISTRY AND GENETICS OF CAPSULAR POLYSACCHARIDE PRODUCTION IN BACTERIA
AUTHOR: ROBERTS I S (Reprint)
CORPORATE SOURCE: UNIV MANCHESTER, SCH BIOL SCI, MANCHESTER M13 9PT, LANCS, ENGLAND (Reprint)
COUNTRY OF AUTHOR: ENGLAND
SOURCE: ANNUAL REVIEW OF MICROBIOLOGY, (1996) Vol. 50, pp. 285-315.
ISSN: 0066-4227.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE

LANGUAGE: ENGLISH
REFERENCE COUNT: 143
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Bacterial polysaccharides are usually associated with the outer surface of the bacterium, They can form an amorphous layer of extracellular polysaccharide (EPS) surrounding the cell that may be further organized into a distinct structure termed a capsule. Additional polysaccharide molecules such as lipopolysaccharide (LPS) or lipooligosaccharide (LOS) may also decorate the cell surface, Polysaccharide capsules may mediate a number of biological processes, including invasive infections of **human** beings. Discussed here are the genetics and biochemistry of selected bacterial capsular polysaccharides and the basis of capsule diversity but not the genetics and biochemistry of LPS biosynthesis (for reviews see 100, 140).

L17 ANSWER 16 OF 22 MEDLINE on STN
ACCESSION NUMBER: 96004600 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7567994
TITLE: Regulation of glycolipid synthesis in HL-60 cells by antisense oligodeoxynucleotides to glycosyltransferase sequences: effect on cellular differentiation.
AUTHOR: Zeng G; Ariga T; Gu X B; Yu R K
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298-0614, USA.
CONTRACT NUMBER: NS-11853 (NINDS)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1995 Sep 12) 92 (19) 8670-4. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 19980206
Entered Medline: 19951023

AB Treatment of the **human** promyelocytic leukemia cell line HL-60 with antisense oligodeoxynucleotides to UDP-N-acetylgalactosamine:beta-1,4-N-acetylgalactosaminyl-transferase (GM2-synthase; EC 2.4.1.92) and **CMP-sialic acid**:alpha-2,8-sialyltransferase (GD3-synthase; EC 2.4.99.8) sequences effectively down-regulated the synthesis of more complex gangliosides in the ganglioside synthetic pathways after GM3, resulting in a remarkable increase in endogenous GM3 with concomitant decreases in more complex gangliosides. The treated cells underwent monocytic differentiation as judged by morphological changes, adherent ability, and nitroblue tetrazolium staining. These data provide evidence that the increased endogenous ganglioside GM3 may play an important role in regulating cellular differentiation and that the antisense DNA technique proves to be a powerful tool in manipulating glycolipid synthesis in the cell.

L17 ANSWER 17 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 95:52787 SCISEARCH
THE GENUINE ARTICLE: QA451
TITLE: SEQUENTIAL-CHANGES IN GLYCOLIPID **EXPRESSION** DURING **HUMAN** B-CELL DIFFERENTIATION - ENZYMATIC BASES
AUTHOR: TAGA S; TETAUD C; MANGENEY M; TURSZ T; WIELS J (Reprint)
CORPORATE SOURCE: INST GUSTAVE ROUSSY, BIOL TUMEURS HUMAINES LAB, CNRS, URA 1156, RUE CAMILLE DESMOULINS, F-94805 VILLEJUIF, FRANCE (Reprint); INST GUSTAVE ROUSSY, BIOL TUMEURS HUMAINES LAB, CNRS, URA 1156, F-94805 VILLEJUIF, FRANCE

COUNTRY OF AUTHOR: FRANCE
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-LIPIDS AND LIPID METABOLISM, (03 JAN 1995) Vol. 1254, No. 1, pp. 56-65. ISSN: 0005-2760.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 44

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have previously reported that **human** B cell differentiation is accompanied by sequential changes in glycosphingolipid **expression**. Pre-B cells contain lacto-series type II chain-based glycolipids and GM3 ganglioside; mature/activated B cells do not synthesize lacto-series compounds but **express** GM3 and globe-series glycolipids (Gb3 and Gb4); terminally differentiated B cells, in addition to these compounds, also contain GM2 ganglioside. At the cell surface, Gb3, Gb3 and GM2 constitute stage-specific antigens. To elucidate the biosynthetic mechanism leading to these modifications we have compared activities of the glycosyltransferases involved in the core structure assembly and the first elongation steps of neo-facto, ganglio- and globe-series glycolipids. These glycosyltransferase activities have been measured in B cell lines and normal B lymphocytes at various stages of differentiation. We first determined the optimal requirements of the four glycosyltransferases which synthesize Lc3, GM3, Gb4 and GM2 glycolipids in B lymphocytes and then tested these enzymes and the Gb3 **synthetase** in the selected B cells. The following results were obtained: beta 1 --> 3 N-Acetylglucosaminyltransferase (Lc3 **synthetase**) has a high activity in pro- and pre-B cells whereas it is undetectable in more differentiated cells; alpha 2 --> 3 sialyltransferase (GM3 **synthetase**) is activated from the pre-B cell stage to the terminally differentiated myeloma cells; alpha 1 --> 4 galactosyltransferase (Gb3 **synthetase**) is only detected in cells representing the late stages of B cell differentiation; beta 1 --> 3 N-Acetylgalactosaminyltransferase (Gb4 **synthetase**) is only found in some lymphoblastoid cell lines, representative of activated B cells whereas the beta 1 --> 4 N-Acetylgalactosaminyltransferase (GM2 **synthetase**) has a high activity in these lymphoblastoid cell lines and in terminally differentiated myeloma cells. These results suggest that the sequential shifts in the three major glycosphingolipid series observed during B cell differentiation are mostly due to sequential activations of the corresponding glycosyltransferases.

L17 ANSWER 18 OF 22 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 95162590 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7858974
TITLE: Combined chemical and enzymatic synthesis of the sialylated non reducing terminal sequence of GM1b glycolylated ganglioside, a potential **human** tumor marker.
AUTHOR: Lubineau A; Auge C; Gautheron-Le Narvor C; Ginet J C
CORPORATE SOURCE: Institut de Chimie Moleculaire d'Orsay, URA CNRS 462, Universite Paris-Sud, Orsay, France.
SOURCE: Bioorganic & medicinal chemistry, (1994 Jul) 2 (7) 669-74. Journal code: 9413298. ISSN: 0968-0896.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950404
Last Updated on STN: 19980206
Entered Medline: 19950320

AB N-Glycolylglucosamine 8 was synthesized in 4 steps from anisal glucosamine, via the new crystalline monochloracetyl derivatives 3, 4 and 7. N-Glycolylneuraminic acid 10 was prepared in 59% yield starting from

pyruvate and a mixture of 8 and its manno epimer 9 in a 2:3 ratio, with immobilized **sialic acid** aldolase. Neu5Gc 10 was converted into **CMP**-NeuGc 11 in the presence of immobilized calf brain **CMP**-sialate **synthetase**. Finally 11 was used as a donor in the transfer to the acceptor beta-D-Gal-(1-3)-beta-D-GalNAc-OBn 12 catalyzed by a preparation of porcine liver (2-3)-alpha-sialyltransferase, roughly purified by a chromatography on Cibacron Blue-agarose. alpha-Neu5Gc-(2-3)-beta-D-Gal-(1-3)-beta-D-GalNac-OBn 13 isolated in 56% yield was deprotected to give the non-reducing terminal sequence of GM1b glycolylated ganglioside, which might be **expressed** in **human** tumors.

L17 ANSWER 19 OF 22 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 92112296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1309720
TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.
AUTHOR: Wessels M R; Haft R F; Heggen L M; Rubens C E
CORPORATE SOURCE: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.
CONTRACT NUMBER: AI07061 (NIAID)
AI22498 (NIAID)
AI28040 (NIAID)
SOURCE: Infection and immunity, (1992 Feb) 60 (2) 392-400.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19990129
Entered Medline: 19920218

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N-acetylneuraminic acid, or **sialic acid**. The presence of **sialic acid** on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a **sialic acid**-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular **sialic acid**. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916 delta E. In contrast to the wild-type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular **sialic acid**, suggesting a defect subsequent to **sialic acid** synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free **sialic acid** to **CMP-sialic acid**: **CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP-sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

	L #	Hits	Search Text
1	L1	1	6783971.pn.
2	L2	2444	"30 contiguous"
3	L3	1	l1 and l2
4	L4	428	fragment same "50 contiguous"
5	L5	0	l1 and l4
6	L6	2759	"50 contiguous"
7	L7	1	l1 and l6
8	L8	12362	synthetase$2
9	L9	32743	"CMP"
10	L10	4051	"sialic acid"
11	L11	88	l8 same l10
12	L12	79	l9 same l11
13	L13	664337	clon$3 or express$3 or recombinant

	L #	Hits	Search Text
14	L14	36	l12 same l13
15	L15	436792	human
16	L17	31778	glycosylat$3
17	L18	0	l14 same l17
18	L16	8	l14 same l15
19	L19	22272	COLEMAN BETENBAUGH
20	L20	6	l12 and l19

	Issue Date	Pages	Document ID	Title
1	20040916	85	US 20040180406 A1	Nucleic acids encoding sialytransferases from C. jejuni
2	20040812	31	US 20040156837 A1	Haemophilus influenzae sialyltransferase and methods of use thereof
3	20040722	39	US 20040142442 A1	Human glycosylation enzymes
4	20040226	259	US 20040038207 A1	Gene expression in bladder tumors
5	20040212	570	US 20040029114 A1	Methods of diagnosis of breast cancer, compositions and methods of screening for modulators of breast cancer
6	20040129	84	US 20040018522 A1	Identification of dysregulated genes in patients with multiple sclerosis
7	20040115	484	US 20040009479 A1	Methods and compositions for diagnosing or monitoring auto immune and chronic inflammatory diseases
8	20031002	31	US 20030186414 A1	Nucleic acid that encodes a fusion protein
9	20030925	26	US 20030180928 A1	Fusion protein comprising a UDP-Galnac 4' epimerase and a galnac transferase

	Issue Date	Pages	Document ID	Title
10	20030821	84	US 20030157658 A1	Polypeptides having beta-1,4-GalNAc transferase activity
11	20030821	84	US 20030157657 A1	Polypeptides having beta-1,3-galactosyl transferase activity
12	20030821	85	US 20030157656 A1	Nucleic acids encoding beta-1,4-GaINAc transferase
13	20030821	84	US 20030157655 A1	Nucleic acids encoding polypeptides with beta-1,3-galactosyl transferase activity
14	20030814	278	US 20030154032 A1	Methods and compositions for diagnosing and treating rheumatoid arthritis
15	20030807	84	US 20030148459 A1	Polypeptides having sialyltransferase activity
16	20030501	78	US 20030082511 A1	Identification of modulatory molecules using inducible promoters
17	20021017	48	US 20020150968 A1	Glycoconjugate and sugar nucleotide synthesis using solid supports
18	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
19	20020926		US 20020137175 A1	Human glycosylation enzymes

	Issue Date	Pages	Document ID	Title
20	20020919		US 20020132320 A1	Glycoconjugate synthesis using a pathway-engineered organism
21	20020509		US 20020055168 A1	Streptococcus suis vaccines and diagnostic tests
22	20020411		US 20020042369 A1	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
23	20020321		US 20020034805 A1	FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHYESIS OF OLIGOSACCHARIDES
24	20020103		US 20020001831 A1	Low cost manufacture of oligosaccharides
25	20040831	46	US 6783971 B2	Human glycosylation enzymes
26	20040420		US 6723545 B2	Polypeptides having .beta.-1,4-GalNAc transferase activity
27	20040302		US 6699705 B2	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
28	20030107		US 6503744 B1	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
29	20020611		US 6403306 B1	Serogroup-specific nucleotide sequences in the molecular typing of bacterial isolates and the preparation of vaccines thereto

	Issue Date	Pages	Document ID	Title
30	20011225		US 6333182 B1	Human glycosylation enzymes
31	20010102		US 6168934 B1	Oligosaccharide enzyme substrates and inhibitors: methods and compositions
32	20000912		US 6117651 A	Expression vectors
33	19980602		US 5759823 A	Ogligosaccharide enzyme substrates and inhibitors: methods and compostions
34	19970114		US 5593887 A	Oligosaccharide enzyme substrates and inhibitors: methods and compositions
35	19951024		US 5461143 A	Oligosaccharide enzyme substrates and inhibitors: methods and compositions

	Issue Date	Pages	Document ID	Title
36	19940111		US 5278299 A	Method and composition for synthesizing sialylated glycosyl compounds

	Issue Date	Pages	Document ID	Title
1	20040722	39	US 20040142442 A1	Human glycosylation enzymes
2	20040226	259	US 20040038207 A1	Gene expression in bladder tumors
3	20040212	570	US 20040029114 A1	Methods of diagnosis of breast cancer, compositions and methods of screening for modulators of breast cancer
4	20030814	278	US 20030154032 A1	Methods and compositions for diagnosing and treating rheumatoid arthritis
5	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
6	20020926	46	US 20020137175 A1	Human glycosylation enzymes
7	20040831	46	US 6783971 B2	Human glycosylation enzymes
8	20011225	47	US 6333182 B1	Human glycosylation enzymes

	Issue Date	Pages	Document ID	Title
1	20040722	39	US 20040142442 A1	Human glycosylation enzymes
2	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
3	20020926	46	US 20020137175 A1	Human glycosylation enzymes
4	20020530	73	US 20020065404 A1	Cytidine monophosphate-sialic acid transporter, and hexosaminidase polynucleotides and polypeptides, and uses based thereon.
5	20040831	46	US 6783971 B2	Human glycosylation enzymes
6	20011225	47	US 6333182 B1	Human glycosylation enzymes

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                   status data from INPADOC
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                   STN Express with Discover!
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 NEWS 13  SEP 27   STANDARDS will no longer be available on STN
 NEWS 14  SEP 27   SWETSCAN will no longer be available on STN
 NEWS 15  SEP 30   STN downtime scheduled October 2-3, 2004

 NEWS EXPRESS  JULY 30 CURRENT WINDOWS VERSION IS V7.01, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
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=> s "sialic acid synthetase?"
L1          195 "SIALIC ACID SYNTHETASE?"

=> s "CMP"
L2        21033 "CMP"

=> s l1 and l2
L3          180 L1 AND L2

=> s human and l3
L4           27 HUMAN AND L3

=> s clon? or express? or recombinant
   5 FILES SEARCHED...
L5      6727337 CLON? OR EXPRESS? OR RECOMBINANT

=> s l4 and l5
L6           14 L4 AND L5

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           10 DUP REM L6 (4 DUPLICATES REMOVED)

=> d 1-10 ibib ab

L7    ANSWER 1 OF 10  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-18268  BIOTECHDS
TITLE:            Producing glycoprotein with animal type sugar chain,
                  comprises introducing gene encoding enzyme that adds sialic
                  acid to non-reducing terminal of sugar chain, and gene of
                  heterologous protein, into plant cell, cultivating plant cell
      ;
                     transgenic plant construction via bacterium-mediated
                     transformation for use in protein production
AUTHOR:           FUJIYAMA K; SEKI T
```

PATENT ASSIGNEE: FUJIYAMA K; SEKI T
PATENT INFO: WO 2004063370 29 Jul 2004
APPLICATION INFO: WO 2004-P 264 15 Jan 2004
PRIORITY INFO: JP 2003-7687 15 Jan 2003; JP 2003-7687 15 Jan 2003
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-561900 [54]

AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) glycoprotein (I) having animal type sugar chain, involves introducing a gene encoding the enzyme that can add sialic acid to the non-reducing terminal of sugar chain, and the gene of heterologous protein, into the plant cell, cultivating the transformed plant cell, and recovering the culture solution of the plant cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) plant cell (II) transformed with the gene that codes **sialic acid synthetase**, **CMP**-**sialic acid synthetase** and/or **CMP**-sialic acid transporter exhibiting saccharide addition mechanism (adding sialic acid to the non-reducing terminal of the sugar chain of glycoprotein), where (II) can take in the precursor of sialic acid or sialic acid, and has a vesicle that allows the uptake of sialic acid; and (2) plant body regenerate from (II).

BIOTECHNOLOGY - Preferred Method: In (M1), the glycoprotein having animal type sugar chain contains a core sugar chain and the external sugar chain, where the core sugar chain essentially comprises several numbers of mannose and acetylglucosamine, while the external sugar chain has a terminal sugar chain moiety containing a non-reducing end galactose. The external sugar chain is the linear or mono, tri or tetra branched sugar chain.

USE - (M1) is useful for producing glycoprotein having animal type sugar chain (claimed).

EXAMPLE - The DNA of the Escherichia coli was used as the template, PCR was performed using the primers having the sequences such as 5'-tttagctcgagacaatgagtaatatatatat-3' and 5'-ttttttctcgagttattattccccctgatttttaaattc-3', the obtained PCR product was digested by XhoI and SalI restriction enzymes, and the neuB fragment was obtained. The DNA of the Nicotiana tabacum cv SRI was used as the template, PCR was performed using the primers having the sequences such as 5'-tttaagtcgacacgatgagagg-3' and 5'-aatcgtcgacccttaactgtc-3', the obtained PCR product was digested by restriction enzymes. The obtained neuB fragment, and the **CMP**-sialic acid transporter (CST) gene were connected, and the target CTS-neuB gene was obtained. The obtained CTS-neuB gene was introduced into the plasmid pBI221, and the vector pBI121-CTS-neuB was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pBI121-CTS-neuB by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of CTS-neuB gene was obtained. The cDNA of the **human** kidney was used as the template, PCR was performed using the primers having the sequences such as 5'-gttactagtatggactcggtggagaaggggccgccacctcc gtcctcaacccgcggggcgaccgtccc-3' and 5'-tgggagctcctatttttggcatgaattatt-3', the obtained PCR product was introduced into the plasmid pBI221. The obtained **expression** cassette was then introduced into pGPTV-HPT, and the pGPTV-HPT-hCSS was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pGPTV-HPT-hCSS by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of HPT-hCSS gene was obtained. The plasmid containing the **expression** cassette of HPT-hCST was also obtained. The plasmids containing the **expression** cassettes of CST-neuB gene and the HPT-hCST gene were digested by XhoI and SpeI enzymes, and both the XhoI/SpeI fragments were introduced into pGEM-T Easy vector, and the plasmid pGEM-T-hCST-CTS-neuB was obtained. To the plasmid pGEM-T-hCST-CTS-neuB gene, the **expression** cassette of HPT-hCSS was introduced, and the plasmid pGPTV-HPT-hCSS-hCST-CTS-neuB was obtained. The pGPTV-HPT-hCSS-hCST-CTS-neuB was introduced into

Agrobacterium tumefaciens LBA4404. The transformed LBA4404 was allowed to infect the cell of tobacco. The infected tobacco cell was cultivated for 7 days, the DNA was isolated from the plant cell, and the presence of the **CMP-sialic acid synthetase** (CSS) and **CMP**-sialic acid transporter (CST) gene was detected. The results showed the presence of CSS and CST genes in the transformed plant cell. Thus the plant cell containing CST and CSS in its genome was obtained. (88 pages)

L7 ANSWER 2 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2004336041 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15238249
TITLE: **CMP-sialic acid synthetase** of the nucleus.
AUTHOR: Kean Edward L; Munster-Kuhnel Anja K; Gerardy-Schahn Rita
CORPORATE SOURCE: Department of Ophthalmology, Case Western Reserve University, Cleveland, OH 4410, USA.
SOURCE: Biochimica et biophysica acta, (2004 Jul 6) 1673 (1-2) 56-65. Ref: 73
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 20040708
Last Updated on STN: 20040818
Entered Medline: 20040817

AB Sialic acids of cell surface glycoconjugates play a pivotal role in the structure and function of animal cells and in some bacterial pathogens. The pattern of cell surface sialylation is species specific, and, in the animal, highly regulated during embryonic development. A prerequisite for the synthesis of sialylated glycoconjugates is the availability of the activated sugar-nucleotide cytidine 5'-monophosphate N-acetylneuraminic acid (**CMP**-NeuAc), which provides the substrate for sialyltransferases. Trials to purify the enzymatic activity responsible for the synthesis of **CMP**-NeuAc from different animal sources demonstrated that the major localisation of the enzyme is the cell nucleus. These earlier findings were confirmed when the murine **CMP**-NeuAc synthetase was **cloned** and the subcellular transport of **recombinant** epitope tagged forms visualised by indirect immunofluorescence. Today, the primary sequence elements that direct murine **CMP**-NeuAc synthetase into the cell nucleus are known, however, information regarding the physiological relevance of the nuclear destination is still not available. With this article, we provide a detailed review on earlier and recent findings that identified and confirmed the unusual subcellular localisation of the **CMP**-NeuAc synthetase. In addition, we take the advantage to discuss most recent developments towards understanding structure--function relations of this enzyme.

L7 ANSWER 3 OF 10 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-06688 BIOTECHDS
TITLE: Manipulating glycoprotein production in insect cell, involves enhancing **expression** of enzymes involved in carbohydrate processing pathway such as N-acetylglucosamine-2 epimerase or **sialic acid synthetase**;
recombinant protein production via plasmid **expression** in host cell for use in diagnosis and therapy
AUTHOR: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A

PATENT ASSIGNEE: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A
PATENT INFO: US 2002142386 3 Oct 2002
APPLICATION INFO: US 2001-930440 16 Aug 2001
PRIORITY INFO: US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-102519 [09]

AB DERWENT ABSTRACT:

NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAc, **sialic acid synthetase**, aldolase, cytidine monophosphate-sialic acid (**CMP**-SA) synthetase or **CMP**-SA transporter, where the **expression** of each (E) is enhanced to above endogenous levels, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP**-SA above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP**-SA, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.

WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B) sequence variants of the amino acid sequence or nucleotide sequence of **human** aldolase, **human CMP**-SA synthetase or SA-synthetase, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP**-SA synthetase is a **human** enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na+, K+-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP**-SA synthetase. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na+, K+-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics and therapeutics.

EXAMPLE - No suitable example given. (88 pages)

L7 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:435294 HCAPLUS
DOCUMENT NUMBER: 135:41800
TITLE: **Recombinant** cells with altered intracellular sialylation pathways and their use in producing glycoproteins
INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Coleman, Timothy A.; Palter, Karen; Jarvis, Don
PATENT ASSIGNEE(S): Human Genome Sciences, Inc, USA; Johns Hopkins University; Temple University; University of Wyoming
SOURCE: PCT Int. Appl., 182 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001042492	A1	20010614	WO 2000-US33136	20001207

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-169839P P 19991209

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human** sialic acid 9-phosphate synthetase which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of sialic acids when the culture medium was supplemented with ManNAc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 10 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001467579 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11479279
TITLE: Molecular **cloning** of a unique **CMP-sialic acid synthetase** that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates.
AUTHOR: Nakata D; Munster A K; Gerardy-Schahn R; Aoki N; Matsuda T;

Kitajima K
CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.
SOURCE: Glycobiology, (2001 Aug) 11 (8) 685-92.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB027414
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010830
Last Updated on STN: 20011015
Entered Medline: 20011011

AB 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a sialic acid (Sia) that is ubiquitously **expressed** in vertebrates during normal development and tumorigenesis. Its **expression** is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including **CMP**-Sia synthetase. Using crude enzyme preparations, it was shown that mammalian **CMP**-Sia synthetases had very low activity to synthesize **CMP**-KDN from KDN and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP**-KDN and **CMP**-N-acetylneuraminic acid (Neu5Ac) (Terada et al. [1993] J. Biol. Chemical, 268, 2640-2648). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA **cloning** of trout **CMP**-Sia synthetase was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme. Based on the Vmax/Km values, the **recombinant** trout enzyme had high activity toward both KDN and Neu5Ac (1.1 versus 0.68 min(-1)). In contrast, the **recombinant** murine enzyme had 15 times lower activity toward KDN than Neu5Ac (0.23 versus 3.5 min(-1)). Northern blot analysis suggested that several sizes of the mRNA are **expressed** in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one **cloned** enzyme has the ability to utilize both KDN and Neu5Ac as substrates efficiently and is useful for the production of **CMP**-KDN.

L7 ANSWER 6 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:189008 BIOSIS
DOCUMENT NUMBER: PREV200200189008
TITLE: Sialylation of the Pasteurella multocida cell surface.
AUTHOR(S): Vimr, E. R. [Reprint author]; Lichtensteiger, C. A. [Reprint author]
CORPORATE SOURCE: University of Illinois at Urbana-Champaign, Urbana, IL, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 141. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Mar 2002
Last Updated on STN: 13 Mar 2002

AB Bacterial pathogens belonging to the Haemophilus-Acthinobacillus-Pasteurella (HAP) group are obligate microparasites of the mammalian oropharynx and can cause severe respiratory or invasive disease in

humans, domestic animals, and wildlife. Sialic acids are ubiquitous components of mammalian cell surfaces and serum glycoconjugates. At least one HAP member, Haemophilus influenzae, has been shown to mimic the host environment by phase-variation of its surface sialic acids. To our knowledge, no other HAP member has been shown to sialylate its cell surface. However, recent DNA sequencing of the Pasteurella multocida genome suggests this bacterium may encode functions for sialic acid catabolism, activation (synthesis of **CMP**-sialic acid), and glycosyl transfer (a2,6-sialyltransferase). To determine if P. multocida is capable of sialylation, the cell-free membrane fractions from two common serotypes (types A and D) were shown to sialylate endogenous acceptor(s) when provided with exogenous **CMP**-(14C)sialic acid. Confirmation that the transferred sialic acid was incorporated into the expected glycoketosidic linkage was obtained by demonstrating sensitivity of the label to digestion with **recombinant** Vibrio cholerae sialidase. The predicted absence of the biosynthetic genes for sialic acid synthesis suggests, as we have shown previously for H. influenzae, that P. multocida acquires free sialic acid from its host and then makes a metabolic decision between catabolism or activation for cell surface sialylation. That P. multocida may synthesize two **CMP-sialic acid synthetases**, one of which is encoded by the last gene of a sialic acid catabolic operon, suggests this HAP bacterium enjoys considerable flexibility in its sialometabolism, potentially accounting for its wide host range.

L7 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:628244 HCAPLUS
DOCUMENT NUMBER: 133:218534
TITLE: **Human** glycosylation enzymes and cDNAs and their use in drug screening, diagnosis, and therapy
INVENTOR(S): Coleman, Timothy A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052136	A2	20000908	WO 2000-US5325	20000301
WO 2000052136	A3	20001228		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000033884	A5	20000921	AU 2000-33884	20000301
EP 1159406	A2	20011205	EP 2000-912096	20000301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6333182	B1	20011225	US 2000-516143	20000301
JP 2002537796	T2	20021112	JP 2000-602748	20000301
US 2002137175	A1	20020926	US 2001-984205	20011029
US 6783971	B2	20040831		
US 2004142442	A1	20040722	US 2004-759277	20040120

PRIORITY APPLN. INFO.:		
US 1999-122409P	P	19990302
US 2000-516143	A3	20000301
WO 2000-US5325	W	20000301

US 2001-984205 A3 20011029

AB The present invention relates to novel **human** glycosylation enzymes and isolated nucleic acids containing the coding regions of the genes encoding such enzymes. Also provided are vectors, host cells, antibodies, and **recombinant** methods for producing **human** glycosylation enzymes. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel **human** glycosylation enzyme polypeptides. Thus, a **human** cDNA encoding a protein with significant sequence homol. to mouse **CMP** N-acetylneuraminic acid synthetase was **cloned** and sequenced. This gene was **expressed** primarily in colon tissue. Another **human** cDNA encoded a protein with significant sequence homol. to C. jejuni cytidine **sialic acid synthetase**. A third **human** cDNA encoding a protein with significant sequence homol. to E. coli N-acetylneuraminic acid aldolase was **cloned** and sequenced. This gene was **expressed** primarily in immune cells and tissues such as primary dendritic cells, monocytes, and bone marrow.

L7 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:628243 HCAPLUS
DOCUMENT NUMBER: 133:233546
TITLE: Engineering of intracellular sialylation pathways for sialylated glycoprotein production
INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Jarvis, Don; Coleman, Timothy A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA; Johns Hopkins University; University of Wyoming
SOURCE: PCT Int. Appl., 145 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2000052135	A2	20000908	WO 2000-US5313		20000301
WO 2000052135	A3	20040108			
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 2000035083	A5	20000921	AU 2000-35083		20000301
JP 2003524395	T2	20030819	JP 2000-602747		20000301
EP 1399538	A2	20040324	EP 2000-913684		20000301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY					
US 2002142386	A1	20021003	US 2001-930440		20010816
PRIORITY APPLN. INFO.:			US 1999-122582P	P	19990302
			US 1999-169624P	P	19991208
			WO 2000-US5313	W	20000301
			US 2000-227579P	P	20000825

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new

carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human** sialic acid 9-phosphate synthetase which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of sialic acids when the culture medium was supplemented with ManNAc.

L7 ANSWER 9 OF 10 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 92112296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1309720
TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.
AUTHOR: Wessels M R; Haft R F; Heggen L M; Rubens C E
CORPORATE SOURCE: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.
CONTRACT NUMBER: AI07061 (NIAID)
AI22498 (NIAID)
AI28040 (NIAID)
SOURCE: Infection and immunity, (1992 Feb) 60 (2) 392-400.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19990129
Entered Medline: 19920218

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N-acetylneuraminic acid, or sialic acid. The presence of sialic acid on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a sialic acid-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular sialic acid. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916 delta E. In contrast to the wild-type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular sialic acid, suggesting a defect subsequent to sialic acid synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free sialic acid to **CMP**-sialic acid: **CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP-sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP**-sialic acid by GBS is an essential step in sialylation of the type III capsular polysaccharide.

L7 ANSWER 10 OF 10 EMBASE
on STN

ACCESSION NUMBER: 92046726 EMBASE
DOCUMENT NUMBER: 1992046726
TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.
AUTHOR: Wessels M.R.; Haft R.F.; Heggen L.M.; Rubens C.E.
CORPORATE SOURCE: Infectious Diseases Division, Harvard Medical School, Beth Israel Hospital,Boston, MA 02115, United States
SOURCE: Infection and Immunity, (1991) 60/2 (392-400).
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N- acetylneuraminic acid, or sialic acid. The presence of sialic acid on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a sialic acid-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular sialic acid. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916ΔE. In contrast to the wild- type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular sialic acid, suggesting a defect subsequent to sialic acid synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free sialic acid to **CMP**-sialic acid: **CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP- sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP**-sialic acid by GBS is an essential step in sialylation of the type III capsular polysaccharide.

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L17 ANSWER 20 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 92046726 EMBASE
DOCUMENT NUMBER: 1992046726
TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.
AUTHOR: Wessels M.R.; Haft R.F.; Heggen L.M.; Rubens C.E.
CORPORATE SOURCE: Infectious Diseases Division, Harvard Medical School, Beth Israel Hospital,Boston, MA 02115, United States
SOURCE: Infection and Immunity, (1991) 60/2 (392-400).
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N- acetylneuraminic acid, or **sialic acid**. The presence of **sialic acid** on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a **sialic acid**-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular **sialic acid**. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916ΔE. In contrast to the wild- type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular **sialic acid**, suggesting a defect subsequent to **sialic acid** synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free **sialic acid** to **CMP-sialic acid**: **CMP -sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP- sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

L17 ANSWER 21 OF 22 MEDLINE on STN
ACCESSION NUMBER: 91152127 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1825612
TITLE: Glycolipids and glycosyltransferases in permanent cell lines established from **human** medulloblastomas.
AUTHOR: Gottfries J; Percy A K; Mansson J E; Fredman P; Wikstrand C J; Friedman H S; Bigner D D; Svennerholm L
CORPORATE SOURCE: Department of Psychiatry and Neurochemistry, University of Goteborg, St. Jorgen Hospital, Hisings Backa, Sweden.
CONTRACT NUMBER: CA 32672 (NCI)
NS 20023 (NINDS)
R37 CA11898 (NCI)
SOURCE: Biochimica et biophysica acta, (1991 Feb 5) 1081 (3) 253-61.
Journal code: 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910428
Last Updated on STN: 19980206
Entered Medline: 19910408

AB Medulloblastoma biopsies are heterogenous and might contain normal brain tissue, which limits the usefulness of such tumor material for biochemical analyses. We have, therefore, examined the gangliosides and their metabolism using the medulloblastoma cell lines. Daoy and D341 Med, cultured both in vitro and as xenografts in nude mice. The ganglioside patterns in the Daoy showed a switch from a high GM2, 70% (mol% of total ganglioside **sialic acid**) and low lactoseries gangliosides (2%) content in monolayer cultures, to a high proportion of lactoseries gangliosides (50%) and virtually no GM2 (1%) in xenografts, but an increased proportion of other a-series gangliosides. The D341 Med showed a similar change regarding the lacto-series gangliosides from 1% in suspension culture to 10% in xenografts. The activity of five glycosyltransferases, GM3, GD3, GM2, GM1 and LA2 synthases, did not parallel the ganglioside patterns and could not account for the noted variations therein. In the Daoy cell line the LA2 synthase as well as the GM2 synthase activity was relatively high in both culture systems, despite the marked difference in the **expression** of GM2 and the lactoseries gangliosides. These results suggest that environmental factors play a crucial role for the in vivo activity of the glycosyltransferases.

L17 ANSWER 22 OF 22 MEDLINE on STN
ACCESSION NUMBER: 89043536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3055198
TITLE: The K1 capsular polysaccharide of Escherichia coli.
AUTHOR: Silver R P; Aaronson W; Vann W F
CORPORATE SOURCE: Division of Bacterial Products, Food and Drug Administration, Bethesda, Maryland.
SOURCE: Reviews of infectious diseases, (1988 Jul-Aug) 10 Suppl 2 S282-6. Ref: 18
Journal code: 7905878. ISSN: 0162-0886.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198812
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19881212

AB Epidemiologic, immunologic, and genetic evidence indicate that the K1 capsular polysaccharide confers invasiveness to Escherichia coli. The capsule, an alpha-2----8-linked homopolymer of **sialic acid** (NeuNAc), provides the bacterium with a physical antiphagocytic barrier. Structural similarities between K1 and **human** tissue components suggest that immune tolerance may also be a factor in pathogenesis of K1 disease. The molecular and genetic events involved in the synthesis and export of the K1 polysaccharide were examined. The **cloned** K1 genes encode at least 12 proteins involved in capsule biosynthesis. These genes appear to be coordinately regulated and functionally clustered. One cluster is associated with the synthesis and activation of NeuNAc and includes the gene encoding **CMP**-NeuNAc **synthetase**. This enzyme catalyzes the activation of NeuNAc to **CMP**-NeuNAc. A second region, encoding five proteins, is associated with translocation of polysaccharide to the

bacterial surface. The K1 polysaccharide is a poor immunogen in **humans**, and an understanding of the key reactions involved in K1 synthesis may help in providing an alternative to anticapsular immunity.

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(FILE 'HOME' ENTERED AT 11:03:05 ON 04 OCT 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004

L1	185254	S	SYNTHETASE?
L2	1	S	"CMP SILAIC ACID"
L3	11	S	"SILAIC ACID"
L4	0	S	L1 AND L3
L5	69303	S	"SIALIC ACID"
L6	603	S	L1 AND L5
L7	21033	S	"CMP"
L8	438	S	L6 AND L7
L9	6727337	S	CLON? OR EXPRESS? OR RECOMBINANT
L10	182	S	L8 AND L9
		E	COLEMAN T A/AU
L11	214	S	E3
		E	BETENBAUGH M J/AU
L12	412	S	E3-E7
L13	613	S	L11 OR L12
L14	7	S	L10 AND L13
L15	4	DUP REM	L14 (3 DUPLICATES REMOVED)
L16	29	S	HUMAN AND L10
L17	22	DUP REM	L16 (7 DUPLICATES REMOVED)